

Higher risk of death among MEN1 patients with mutations in the JunD interacting domain: a Groupe d'étude des Tumeurs Endocrines (GTE) cohort study

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Multiple endocrine neoplasia syndrome type 1 (MEN1), which is secondary to mutation of the *MEN1* gene, is a rare autosomal-dominant disease that predisposes mutation carriers to endocrine tumors. Although genotype–phenotype studies have so far failed to identify any statistical correlations, some families harbor recurrent tumor patterns. The function of MENIN is unclear, but has been described through the discovery of its interacting partners. Mutations in the interacting domains of MENIN functional partners have been shown to directly alter its regulation abilities. We report on a cohort of MEN1 patients from the Groupe d'étude des Tumeurs Endocrines. Patients with a molecular diagnosis and a clinical follow-up, totaling 262 families and 806 patients, were included. Associations between mutation type, location or interacting factors of the MENIN protein and death as well as the occurrence of MEN1-related tumors were tested using a frailty Cox model to adjust for potential heterogeneity across families. Accounting for the heterogeneity across families, the overall risk of death was significantly higher when mutations affected the JunD interacting domain (adjusted HR = 1.88; 95%-CI = 1.15–3.07). Patients had a higher risk of death from cancers of the MEN1 spectrum (HR = 2.34; 95%-CI = 1.23–4.43). This genotype–phenotype correlation study confirmed the lack of direct genotype–phenotype correlations. However, patients with mutations affecting the JunD interacting domain had a higher risk of death secondary to a MEN1 tumor and should thus be considered for surgical indications, genetic counseling and follow-up.

INTRODUCTION

Multiple endocrine neoplasia syndrome type 1 (MEN1, OMIM 131100) is an autosomal dominant disorder that predisposes carriers to endocrine tumors (1). The prevalence has been estimated between 1 out of 30 000 and 1 out of 100 000 (2). The tumors mainly develop from endocrine tissues and may arise from parathyroid glands (90–100%), the pancreas (50–70%), pituitary gland (20–40%), adrenal glands (20–40%) and at a lower frequency from the bronchi and thymus (<10%) (3). MEN1 syndrome is secondary to germline mutations of the *MEN1* gene, mapped to the 11q13 locus and encoding for the MENIN protein (4). The function of the MENIN protein remains

unclear. Two types of functional domains are described: Nuclear localization sequences (NLS) and the MENIN domain (5,6). No mutational hot spots have been defined, and all the protein domains are affected (7–9). In contrast with other genetic diseases, no obvious genotype–phenotype correlations have been established so far (10,11). The existence of familial clusters of thymic tumors raised the issue of possible heterogeneity across families. Mild/late MEN1 phenotypes exist, as well as family phenotypes with prominent features of prolactinomas, of isolated hyperparathyroidism or with more aggressive gastro-pancreatic neuroendocrine tumors (NETs) (12). The severity of the disease is at present unpredictable

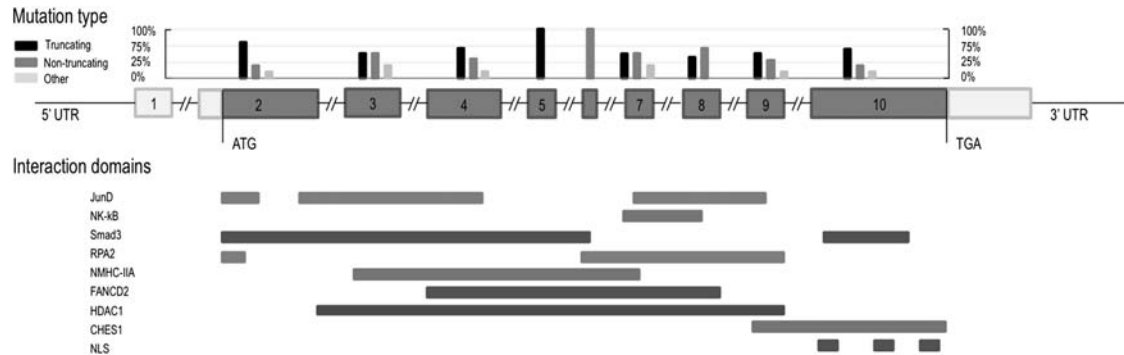


Figure 1. Schematic representation of the genomic organization of the MEN1 gene. The human MEN1 gene consists of 10 exons that span more than 9 kb of genomic DNA and encodes a 610 amino acid protein. The start (ATG) and stop (TGA) codons in exons 2 and 10, respectively, are indicated. Exon 1, the 5' part of exon 2, and the 3' part of exon 10 are untranslated (indicated by open boxes). (A) 5' and 3' segmentation of the gene product distinguishes two equivalent parts from exons 2 and 3 versus exons 8–10, respectively. (B) The distribution of mutation types is represented showing truncating mutations (black boxes) and non-truncating mutations (open boxes). (C) The MENIN interacting partners are positioned (adapted from 9).

(13). Pancreatic tumors and thymic tumors may unfortunately turn into aggressive tumors with metastatic spread (4,14,15). Nevertheless, nobody knows whether the severity of such tumors might be related to specific mutations or not. Gender has been shown to be a significant modifying factor for disease expressivity. Indeed, we previously showed that women were more prone to pituitary lesions and males to gastrinomas (16–19). Thymic tumors were thought to occur almost exclusively in men in many studies (20,21). Nevertheless, a recent large Japanese collection of MEN1 data did not confirm what used to be considered a general rule (22).

Previous phenotype–genotype correlation studies on smaller population samples have been published (2,7,23). The largest study published to date counted 258 patients (23). Such studies require larger numbers of patients because of the various types of mutations. In addition, MEN1 exhibits a high, but progressive penetrance during the lifespan. Therefore, correlation studies need to take into account the time-dependent expressivity and expected heterogeneity across families using appropriate statistical techniques (10). Finally, MENIN interacts with various partners (9,24). Mutations affecting the interacting domain of MEN1 could interfere with and abolish interactions with functional partners (Fig. 1) (24–28). Loss of interaction (LOI) with MENIN may modify disease expressivity (28). Various functions such as (i) regulation of transcription with JunD, MLL-HMT and others, (ii) stabilization of the double DNA strand and of the genome and (iii) regulation of cell cycle and division (9) have been described for each partner. Therefore, the absence of physiologic interactions also needs to be evaluated in a MEN1 genotype–phenotype study.

This study aimed to assess associations between the phenotypic manifestation of the disease and both the mutation types and the locations in interacting domains. This MEN1 genotype–phenotype study was based on a cohort of 806 patients from 262 unrelated families with molecular diagnosis collected through the ‘Groupe d’étude des Tumeurs Endocrines (GTE)’.

RESULTS

Overall, 262 mutations were diagnosed in the 806 patients. Among these, 463 patients (59%) carried a truncating mutation

and 142 (18%) a non-truncating mutation (Table 1). The gender ratio (female/male) was 1:1. Median follow-up was 47 years (Inter-quartile ratio = 30–59 years; ranging from 3 to 86 years). Seventy-five patients were under 18 years old (9%), and at their last follow-up, a total of 118 patients (15%) had not had a tumor diagnosed. Overall, 1522 tumors were diagnosed (1.9 tumors per patient). The heterogeneity across families was statistically significant in 77% of cases (65 out of 84 tests) (Supplementary Material, Fig. S1) and followed a log normal distribution (Fig. 2). Thus, subsequent estimates of genotype–phenotype association accounted for this heterogeneity across families. No mutational hot spot was evidenced. There was no statistically significant association between the genotypic criteria ‘truncating or non-truncating mutation’ and the phenotypic criteria. As well, no statistically significant association was found between the genotypic criteria ‘5’ versus 3’ mutation’ and the different phenotypic criteria. In contrast, five tests related to the LOI of MENIN with its partner were statistically significant before correction (Table 2). After correction, only JunD-LOI remained significantly associated with an increased overall risk of death (P -value = 0.008; Q -value = 0.048). Gender was significantly associated with the occurrence of pituitary tumors in females (Q -value < 10^{-4}) and thymic tumors in males (Q -value = 0.010). A higher risk of death was observed for men than for women (Q -value = 10^{-4}). Patients with JunD-LOI had a higher risk of MEN1-related death after adjustment for gender [HR = 1.99 (CI-95% = (1.08; 3.63))] (Table 3). More precisely, JunD-LOI was associated with decreased survival in the subgroup of patients who died from MEN1-related tumors [HR = 2.34 (CI-95% (1.23; 4.43))] (Table 3 and Fig. 3). Conversely, JunD-LOI was not associated with either deaths related to MEN1 non-cancer etiologies (P = 0.690), or with non-MEN1-related deaths (P = 0.920). After excluding the 23 patients with thymic tumors, patients with JunD-LOI still presented a higher risk of death [HR = 1.73 (CI-95% (1.03; 2.93)); P = 0.020].

DISCUSSION

So far, no direct correlation between genotype and phenotype has been established in MEN1 disease (12). This new

Table 1. Descriptive statistics of the GTE cohort

			Phenotypic criteria						Death
			Tumor type						
			Parathyroid	Pituitary	Adrenal	Pancreatic	Bronchial	Thymic	
Genotypic criteria	Total (<i>n</i> = 806)		645	260	145	415	34	23	93
	Gender								
	Men	(<i>n</i> = 373 out of 806)	292 (46%)	93 (36%)	69 (48%)	198 (47.7%)	16 (47%)	22 (96%)	59 (63%)
	Women	(<i>n</i> = 433 out of 06)	353 (54%)	167 (65%)	76 (52%)	217 (52.3%)	18 (53%)	1 (4%)	34 (37%)
	Mutation type ^a								
	Truncating	(<i>n</i> = 473 out of 806)	374 (58%)	135 (52%)	89 (61%)	243 (59%)	14 (42%)	12 (52%)	52 (56%)
	Non-truncating	(<i>n</i> = 142 out of 806)	109 (17%)	42 (16%)	26 (18%)	73 (17%)	9 (26%)	3 (13%)	17 (18%)
	Other	(<i>n</i> = 191 out of 806)	162 (25%)	83 (32%)	30 (21%)	99 (24%)	11 (32%)	8 (35%)	24 (26%)
	Mutation localization ^b								
	5′	(<i>n</i> = 178 out of 623)	142 (43%)	61 (33%)	27 (23%)	95 (30%)	7 (29%)	6 (37%)	17 (24%)
	3′	(<i>n</i> = 322 out of 623)	251 (57%)	90 (50%)	55 (47%)	163 (51%)	15 (62%)	8 (50%)	43 (62%)
	Interaction domain ^b								
	JunD	(<i>n</i> = 298 out of 800)	251 (39%)	103 (40%)	91 (64%)	163 (40%)	12 (36%)	15 (65%)	46 (50%)
	NF-kB	(<i>n</i> = 116 out of 786)	95 (15%)	35 (14%)	19 (14%)	52 (13%)	5 (15%)	4 (18%)	12 (13%)
	Smad3	(<i>n</i> = 479 out of 800)	392 (61%)	155 (60%)	82 (57%)	239 (58%)	12 (36%)	13 (56%)	50 (54%)
RPA2	(<i>n</i> = 247 out of 800)	203 (32%)	78 (30%)	43 (30%)	133 (32%)	12 (36%)	8 (35%)	30 (32%)	
NMHC II-A	(<i>n</i> = 219 out of 788)	182 (29%)	71 (31%)	37 (27%)	109 (27%)	4 (12%)	8 (35%)	26 (28%)	
FANCD2	(<i>n</i> = 219 out of 788)	178 (28%)	67 (27%)	42 (30%)	106 (26%)	7 (21%)	5 (23%)	20 (22%)	
HDAC1	(<i>n</i> = 403 out of 800)	328 (51%)	137 (53%)	75 (52%)	210 (51%)	12 (36%)	15 (65%)	48 (52%)	
CHES1	(<i>n</i> = 304 out of 786)	242 (39%)	92 (37%)	49 (35%)	141 (35%)	12 (36%)	8 (35%)	36 (39%)	
NLS	(<i>n</i> = 89 out of 786)	77 (12%)	32 (13%)	11 (8%)	39 (10%)	3 (9%)	3 (14%)	9 (10%)	

^aTruncating = FS+NS; Non-truncating = IF+MS; Other = SP+other.
^bDifference in the total number of patients (n = 806) is due to missing values. Tumor types considered were the most frequent ones.

phenotype–genotype correlation study reports on the largest cohort of MEN1 patients to date. This study used a time-to-event statistical analysis and considered for the first time the effect of mutations located in the interacting domains. Accounting for the heterogeneity across families, mutations affecting the interacting domain with JunD were associated with a significantly higher risk of death secondary to MEN1-related cancers.

The MEN1 GTE cohort has already been described and is deemed representative of MEN1 disease in Western Europe (17–20,29–31) with no major difference in terms of lesion prevalence when compared with the independent German cohort (23). The GTE cohort had a median follow-up observation time of 47 years and thus allowed to study reliably the age-dependent expressivity of MEN1 disease with time-to-event techniques (17–20,29–33). The time-to-event approach has already been used once on a limited patient sample and without considering the potential heterogeneity across families (23).

Testing the statistical independence of the observations within families (i.e. the heterogeneity across families) was a prerequisite for the subsequent analyses. Indeed, significant heterogeneities were identified for most tumors as well as for survival and led us to account for this inter-familial variability. However, a positive test could not identify a family particularly at risk of developing a given lesion. Such results require further studies and raise the issue of allelic modifying factors of the MENIN protein that may explain the variable penetrance and expressivity of the disease.

In addition, we also used a false discovery rate (FDR) correction procedure (34) to account for multiple testing. The Benjamini and Hochberg method was chosen for its ability to control the FDR while staying adequate in case of

independence between tests and when a positive dependency of the tests could be hypothesized (35,36). Indeed, a significant genotype–phenotype association does not generally provide any information about the effects of other distinct genotypes. In case of overlapping interacting domains, similar results would be expected for the genotype–phenotype associations leading to a positive dependency between tests. Using an appropriate methodology in the largest cohort of MEN1 patients with confirmed mutations, we (i) analyzed the impact of mutations located in the interacting domain resulting in a putative LOI while accounting for heterogeneity across families and (ii) we conducted a survival study considering death as a phenotypic aspect of the disease.

Regarding the phenotypic expression of the six tested tumor types, no genotypic correlation was found. This was in accordance with previous results from the literature (23) (Table 4). As already pointed out by the GTE, an association was found between gender and phenotypic criteria such as the development of pituitary tumors, thymic tumors (19) and survival (21). The prevalence of pituitary adenomas is greater in female MEN1 patients than in the non-MEN1 population. Thymic tumors are known to occur almost exclusively in male patients, at least in Western Europe and the USA (21,37). These gender-related differences were taken into account in the adjustment computations.

The function of the MENIN protein was ascertained by various approaches, one of which was the identification of MENIN partners. When the transcription factor JunD is deprived of MENIN, it switches from growth suppressor to growth promoter (26). The hypothesis that point mutations could affect the interaction with known functional partners was supported by structural or functional data available for some of MENIN partners such as MLL, JunD and others

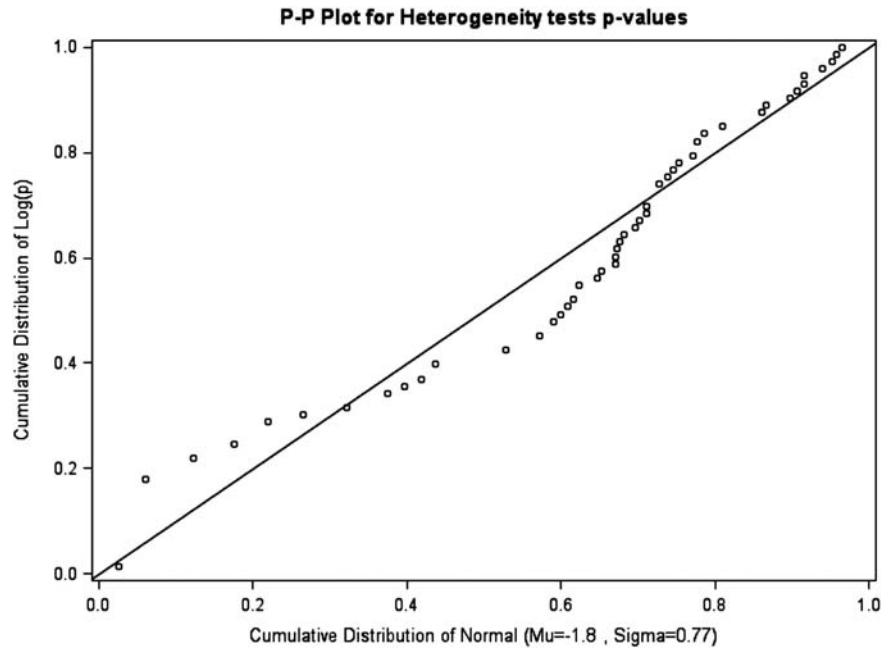


Figure 2. P–P plot for *P*-values of the heterogeneity tests across families in this study (GTE cohort-*n* = 806, 84 heterogeneity tests). Observed *P*-values approximately display a log-normal distribution for the 84 heterogeneity tests in this study.

Table 2. Genotype–phenotype correlations using the frailty model of Cox-hazard ratio and FDR correction in the GTE cohort-*n* = 806–2011

Phenotype	Genotype	HR (95% CI)	<i>P</i> -values	FDR correction (<i>Q</i> -value)
Death	JunD-LOI	1.88 (1.15; 3.07)	0.008*	0.048**
Bronchial tumor	Smad3-LOI	0.37 (0.18; 0.78)	0.009*	0.1
Thymic tumor	JunD-LOI	3.01 (1.15; 7.86)	0.024*	0.14
Pancreatic tumor	CHES1-LOI	0.78 (0.61; 0.99)	0.04*	0.54
Bronchial tumor	NMHC-LOI	0.33 (0.11; 1.003)	0.05	0.3

Twelve genotype criteria correlated with 7 phenotypic criteria. The five most significant results are shown, without (*P*-values) and with the FDR correction (*Q*-value). Significant tests are shown without (*) and with the FDR correction procedure (**). Further results in the text are corrected *Q*-value values.

(24,38,39). We generalized this hypothesis to all the factors with known interaction domains because *in vitro* and *in vivo* models demonstrated that some MENIN mutations affected the functions of MENIN protein with regard to its partners (25). Functional studies on cancer cells revealed that the MENIN protein played a crucial role in repressing the transcription of pro-oncogenic factors such as JunD or MLL (28,39). The MLL protein was a promising interacting factor of MENIN. Unfortunately, the MLL interacting domain with MENIN was not published as a primary amino acid sequence and was, thus, not included as a genotypic factor, but its three-dimensional model suggests the existence of an interacting domain binding the MENIN protein similar to that for JunD (38,39).

When mutated, the JunD interacting domain was associated with a significant decrease in overall survival. This result is of interest because of the already known pro-oncogenic functions

of JunD in various cancer types (40,41). MENIN protein lowers the transcriptional activity when binding to JunD in targeted tissues, with secretory and non-secretory consequences (24,42–44). In this cohort, survival was significantly lower among patients carrying mutations affecting the JunD interacting domain, with a 2-fold increased risk of dying from MEN1-related cancers. JunD-LOI mutations were not associated with either other causes of death or with any peculiar phenotypic expressivity. This aggressiveness was not only related to thymic tumors because the risk of dying remained significant after their removal from the computations. Nonetheless, JunD is not the only factor to bind in this region, and MLL-LOI might be implicated in tumor aggressiveness when such mutations are diagnosed. The conclusion drawn from this work is that a mutation located in the area involved in JunD interactions may decrease survival in MEN1-related tumors. To confirm this hypothesis, functional studies measuring JunD or MLL activity should be conducted in patients with a mutation located in the codon position 1–40, 139–242 and 323–428. Although the underlying mechanisms remain unclear, the identification of a group of at-risk mutations in the *MEN1* gene is relevant in terms of clinical implications for counseling, follow-up rules and surgical indications.

In the GTE cohort, half of the MEN1-cancer-related deaths were due to pancreatic NETs. The indication for surgery in pancreatic NET is difficult because several factors need to be considered. These include: the size of the largest tumor (2 cm or more) (24), the ability to control hormonal secretions, the progression of the tumor, a high mitotic index in the diagnostic biopsy, the physiologic status of the patient and the patient's motivation for surgery (SFNGE guidelines) (33). When there are doubts about the usefulness of surgery, the JunD-LOI status may be an important additional element for decision making. International guidelines advise a regular

Table 3. Impact of JunD-LOI on the risk of death (frailty Cox proportional model) in the GTE cohort—*n* = 806–2011

Association between JunD-LOI and causes of death [number of patients (%)]	JunD-LOI 298 (37.3%)	Unadjusted HR HR (95% CI)	<i>P</i> -value	Adjusted on gender HR HR (95% CI)	<i>P</i> -value
MEN1-related death (cancer or other complications)	34 (11%)	2.07 (1.13; 3.78)	0.01	1.99 (1.08; 3.63)	0.01
MEN1-non-cancer related death (secretion complications)	4 (1%)	0.82 (0.24; 2.83)	0.7	0.81 (0.23; 2.80)	0.7
All cancers related death (MEN1 or others)	34 (11%)	2.07 (1.18; 3.79)	0.008	2.04 (1.16; 3.60)	0.01
MEN1-cancer-related death	30 (10%)	2.42 (1.27; 4.61)	0.005	2.34 (1.23; 4.43)	0.006
Thymic tumor-related death	9 (3%)	2.94 (0.81; 10.67)	0.08	2.56 (0.71; 9.18)	0.15
Non-MEN1 cancer death	4 (1%)	0.94 (0.27; 3.20)	0.9	0.94 (0.27; 3.22)	0.9

Among the 93 patients who died during follow-up, 46 patients were diagnosed with a JunD-LOI (50%). The cause of death was missing for six patients. The 800 patients are compared with the causes of death of the 93 patients depending on their JunD-LOI status.

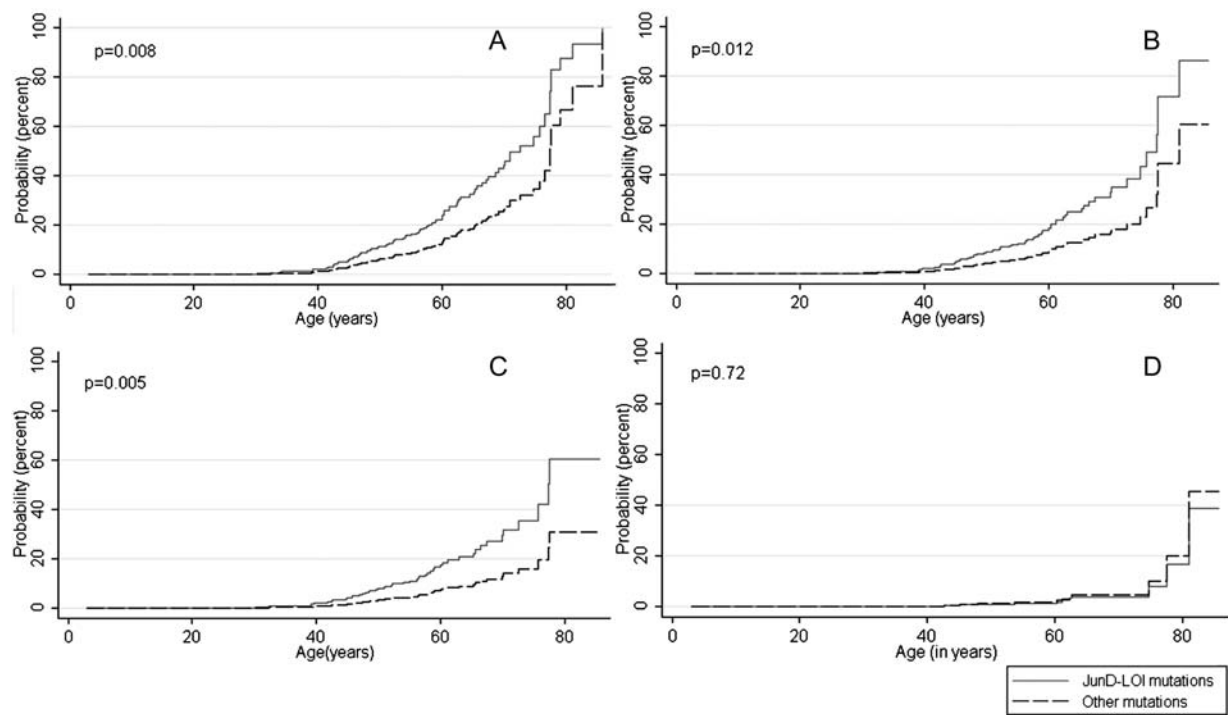


Figure 3. Survival analysis in the GTE cohort, considering the JunD-LOI status and cause of death. Abscises represent the age at the event or at last follow-up. Ordinates represent the probability of tumor occurrence (percentage). (A) Overall survival was significantly correlated with JunD-LOI. Three groups were created: (B) risk of death from a MEN1 cause, (C) risk of death from a MEN1 cancer and (D) risk of death from a non-MEN1 cancer. Significant results are highlighted on the plots. Patients of the GTE cohort were significantly more likely to die from MEN1 cancer than from any other cause.

abdominal imaging follow-up every 3–5 years. We would recommend a reinforced follow-up program in patients who are genetically at risk because of a JunD-LOI mutation.

In conclusion, this study demonstrates the existence of at-risk mutations within the codons involved in the interaction between MENIN and JunD. In this study, JunD-LOI was significantly associated with a higher risk of death. This reduced survival was secondary to pleiotropic MEN1 cancers, suggesting that the tumors are more aggressive. Specific recommendations for pancreatic surgery and follow-up might be pertinent in patients carrying a JunD-LOI mutation. The existence of an heterogeneity across families in MEN1 syndrome is now established, suggesting the importance of genetic modifying factors in the variable expressivity of MEN1 syndrome.

Further studies on the modifying factors implicated in heterogeneity across families of MEN1 syndrome might be a key to understand the variable expressivity of MEN1 syndrome.

POPULATION AND METHODS

Population

The GTE network for MEN1, created in February 1991, brings together clinical centers in France and Belgium together with the four genetics laboratories in charge of diagnosis. In 2011, the GTE cohort for MEN1 included a total of 912 patients from 278 regularly followed families (17–20,29–31). To be included in this genotype–phenotype correlation study,

Table 4. Previous genotype–phenotype studies in MEN1 syndrome

Study	Sample size Total Kindred		Phenotypic criteria	Genotypic criteria	Statistical analysis Limits	Advantages
Kouvaraki <i>et al.</i> (2)	109	24	5 tumor types ^a 6 PET subtypes	13 mutations available for analysis 4 mutation types Mutation localization ^b	Age not taken into account No correction procedure	Direct comparisons (Fisher's exact test and χ^2)
Wautot <i>et al.</i> (7)	170	170	Familial tumor accumulation	Grouped mutation types ^c	Age not taken into account	Direct comparisons (χ test)
Machens <i>et al.</i> (23)	258	116	5 tumor types ^a Age at tumor diagnosis Malignancy	Grouped mutation type ^c	Direct comparisons (ANOVA, χ) No correction procedure	Age account Time-dependent Kaplan– Meyer analysis (log rank test)
Present study	806	262	5 tumor types ^a Age at tumor diagnosis Death	Mutation localization ^b Grouped mutation type ^c		Age account Time to event analysis Heterogeneity across families tested Survival analysis

Three genotype–phenotype studies were available in the literature, with various approaches.

^aPET, pancreatic endocrine tumors, parathyroid, pituitary, adrenal and carcinoid (bronchial and thymic) tumors.

^bReferring to exons 2, 9, 10 and others.

^cReferring to truncating, non-truncating and other mutations.

Table 5. JunD status of the 51 patients with a MEN1 cancer-related death in the GTE cohort— $n = 806$ –2011

Tumor type	Total $n = 51$	JunD-LOI $n = 30$	Others $n = 21$
Non-secreting pancreatic NET	14	7	7
Secreting pancreatic NET	12	8	4
Gastrinoma	8	5	3
Glucagonoma	2	1	1
Insulinoma	1	1	0
Vipoma	1	1	0
Thymic tumor	11	8	3
Bronchial NET	6	2	4
Ependymoma	2	2	0
Adrenal tumor	2	1	1
Pituitary adenoma	1	1	0
Gastric NET	1	0	1
Unknown origin	2	1	1

NET, neuro endocrine tumor.

symptomatic or non-symptomatic MEN1 patients needed a genetic MEN1 diagnosis and available data on phenotypic expression. Overall, 823 patients had a genetic diagnosis. Among these, 17 patients (2.1%) from 16 different families were excluded from the analysis because of missing information. The analysis finally included 806 patients from 262 families (Table 1).

Genotypic factors

The various genotypic abnormalities were grouped according to the following classifications: (i) depending on the functional aspect of MENIN: truncating (nonsense, frame-shift mutations) versus non-truncating mutations (missense, in-frame insertions or deletions) and versus the remaining mutations (splicing and intronic mutations, intragenic and genic rearrangements), (ii) mutations in 5' versus 3': the 610 amino acids of the MEN1 coding sequence were divided into 3 parts; the 5'

and 3' parts encoded, respectively, by exons 2 and 3 and 8–10 were considered and (iii) mutations affecting an interacting domain, causing a LOI with the functional partners. Mapping of the interacting domains has already been published and reviewed (9). The following functional or interacting domains were considered [protein name (codon positions)]: JunD (1–40, 139–242 and 323–428), NF- κ B (305–381), Smad3 (40–278 and 477–610), Pem (278–476), NM23H1 (1–486), RPA2 (1–40 and 286–448), NMHC II-A (154–306), FANCD2 (219–395), mSin3A (371–387), HDAC1 (145–450), ASK (558–610), CHES1 (428–610) and NLS (479–497, 546–572 and 588–608) (Fig. 1). When the locations of mutations were compared, analyses were performed with all types of mutations. For further analyses, the causes of death were categorized as: (i) secondary to a cancer of the MEN1 spectrum (i.e. involving the pancreas, parathyroid glands, pituitary gland, adrenal glands, bronchi and thymus), (ii) secondary to a non-MEN1-spectrum cancer and (iii) not secondary to cancer (Table 5).

Statistical methods

Time-to-event techniques were used to identify genotype–phenotype correlations. Events of interest were: (i) death from any cause and (ii) first occurrence of each of the six main types of MEN1 lesions (i.e. involving the pancreas, parathyroid glands, pituitary gland, adrenal glands, bronchi and thymus). Events with fewer than 10 occurrences in the cohort were excluded (stomach and brain). Birth was considered the baseline. Patients who did not experience an event of interest during their follow-up were censored at the date of their last follow-up. For each genotype–phenotype correlation, a frailty Cox's proportional hazards model was used with the family as a frailty component (45). This model tests for both heterogeneity across families (likelihood ratio test) and genotype–phenotype associations and can be adjusted for other covariates when necessary. The prognostic role of the 12 genotypic profiles for each of the 7 phenotypic criteria

(survival in addition to 6 types of tumor) was assessed using a univariate frailty model. This analysis led to 84 genotype–phenotype association tests ($84 = 12 \times 7$). To control the risk of false positive results due to the multiplicity of tests, the Benjamini and Hochberg FDR method was used (34–36). The FDR is defined as the expected proportion of false positives among all (significant) discoveries. Thus, for each association test, we calculated a Q -value representing the minimum FDR at which the statistical test may be considered as a true positive (i.e. significant taking into account the multiplicity of tests) (46). A result was deemed false positive when the corresponding Q -value was above 0.05. In addition, the heterogeneity across families was assessed for each of these tests, and a descriptive analysis was performed through a P–P plot (Fig. 2). The association tests were also adjusted on gender for phenotypic criteria when gender was thought to be a confounding factor. In addition, interactions between gender and other genotypic profiles were systematically checked. Cumulative probabilities of lesion occurrence or death were estimated using the Breslow estimator. Stata software version 11 (Stata Corp., College Station, USA) was used for frailty models and probability estimates, and SAS software version 9.3 (SAS institute, Cary, USA) was used for all other analyses.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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